This theory presumes that hydrostatic pressure, either blood or tubular or interstitial, is held constant in the tubular sections of the nephron; these constant pressures are presumed to play no role in the process of absorption. Rather, the differential absorptive process is governed entirely by fluctuating oncotic pressures and cellular metabolic processes.

Summary. 1. When the renal vein is completely occluded, the intrarenal pressure increases about 3-fold, being elevated to the simultaneous pressure in the vein on the renal side of the occlusion. Usually this pressure is somewhat less than the simultaneous diastolic blood pressure. When the vein is partially occluded, intrarenal pressure does not change until the simultaneous pressure in the vein on the renal side of the occlusion rises to the original intrarenal pressure. At this point, as the occlusion is increased, the two pressures rise together and always have the same value. 2. The hypothesis is proposed that the pressure inside the renal peritubular capillaries, venules, uriniferous tubules and lymphatics is normally just above intrarenal pressure, *i.e.* at 25 mm Hg. This situation, it is postulated, permits the process of reabsorption to take place freed from the influence of random or pathological fluctuations in vena cava pressure. Oncotic pressures and cellular metabolic processes, therefore, are presumed to be the primary determinants of differential reabsorption.

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Resistance Lowering Effect of Human Respiratory Tract Mucin.* (18630)

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Although hog gastric mucin has been extensively used since 1932(1) to lower the resistance of laboratory animals to bacteria, the possible role of excessive mucus accumulations on the defense mechanisms of man has received little attention. Because of the commercial availability of hog gastric mucin, this material has been used almost exclusively as an aid in producing experimental infections. The senior author in unpublished work has used human respiratory tract mucin in a few trials with results quite similar to those obtained with gastric mucin. It seemed desirable to confirm and extend these earlier unpublished observations with human respiratory tract mucin since there are many clinical situations where excessive mucus could be significant in lowering the resistance of man.

The human mucin used in this study was obtained from sputum collected from patients with varying lung conditions (lung neoplasms, bronchiectasis, lung abscess, asthma, suppurative pneumonitis). The different samples of sputum were homogenized together in a CO_2 atmosphere by means of a Waring blendor. The mucin was then sterilized by heating at 80°C for ten minutes. Sterility of the mucin was controlled by culture on a blood agar plate.

Intra-bronchial inoculations in rats. A small vertical incision was made through the skin and muscles overlying the trachea to aid in passing a number 5 French ureter catheter into a main bronchus. Although the exposed trachea was not opened, a clear view was had of the descending catheter. In this manner, passage of the inoculum into the esophagus was avoided with certainty. After inoculation, the skin incision was closed with a single suture. Four series of experiments were done, each consisting of 5 groups of 5 rats each. A suspension of pneumococci, type I, was suspended in different menstrums and 0.2 cc was injected intra-bronchially into each animal. Each rat received an inoculum of 10^{-4}

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1. Nungester, W. J., Wolf, A. A. and Jourdonais, L. F., PROC. SOC. EXP. BIOL. AND MED., 1932, v30, 120.

TABLE I. Effect of Human Respiratory Tract Mucin in Lowering Resistance of Lungs of Rats to Pneumococcus Pneumonia.

Inoculum	No. of animals				
	Inoculated	Pneumonia involving lobe			
		>50%	20–50%	<20%	0%
		%	%	%	%
Pne. I 10^{-4} ml in human mucin	20	60	20	20	
Pne. I 10^{-4} ml in hog gastric mucin	20	80	20		
Pne. I 10^{-4} ml in saline	20				100
Human mucin alone	20			100	
Hog gastric mucin alone	20			100	

ml of a culture having a plate count of approximately 10^8 per ml. After 48 hours the animals were sacrificed. Table I presents the results which clearly indicate that human mucus was approximately as effective as hog gastric mucin in lowering the resistance of the rat lung to pneumococcus pneumonia. In order to eliminate such factors as atelectasis from a consideration of the resistance lowering properties of human mucus, the intrabronchial experiments in rats were discontinued in favor of intraperitoneal inoculation of mice. It is generally accepted that the main effect of mucin in intraperitoneal infections involves the phagocytic cells and possibly the intracellular destruction of the bacteria(2,3). There is essentially no evidence that mucin increases the virulence of bacteria, a careless implication originally extended by the senior author.

Intraperitoneal inoculations in mice. A culture of a group C hemolytic streptococcus was used for the experiments, designed to evaluate the resistance lowering effect of human mucin and its fractions when injected intraperitoneally with the test organism. Four series of experiments were done. Each consisted of eleven groups of 5 mice. Three different dilutions of streptococci were suspended in human mucin so that the inoculum 0.25 ml contained 10^{-4}, 10^{-6}, or 10^{-8} of the original culture suspended in human or hog mucin or saline. Two control groups of mice received human respiratory mucin or hog gastric mucin alone. Table II presents the typical results of a test in this series of experiments. It is apparent that human

TABLE II. Resistance Lowering Properties of Human Respiratory Tract Mucin Tested by Intraperitoneal Inoculation of Mice.

Inoculum Streptococcus group C	Suspended in		
	Human mucin	Hog gastric mucin	Saline
10^{-4}	5/5*	5/5	2/5
10^{-6}	5/5	5/5	0/5
10^{-8}	1/5	2/5	0/5

* Fraction $= \frac{\text{No. dead at 4 days}}{\text{No. inoculated}}$.

Mucin solutions alone were not lethal.

mucin has a resistance lowering effect almost equal to that of hog gastric mucin. Morgan and King(5) isolated from crude gastric mucin a material identical with the human blood group A factor. Landy and Batson(6) have recently shown a correlation between the resistance lowering action of hog gastric mucin and its content of human blood group A antigen. This suggested a comparison of fractions of hog gastric and human mucin for resistance lowering properties and their content of blood group A antigen.

Fractionation procedures. The fractions of hog gastric mucin† were obtained essentially by one of the methods of Morgan and King (5). This consists of extraction of the material with 90% phenol, centrifuging and then treatment of the supernate with ethanol to a given concentration. The precipitate formed at this ethanol concentration is centrifuged, washed

2. Nungester, W. J., Jourdonais, L. F., and Wolf, A. A., *J. Inf. Dis.*, 1936, v59, 11.

3. McLeod, C., *Am. J. Hyg.*, Sec. B., 1941, v34, 41.

4. Olitzki, L., *Bact. Rev.*, 1948, v12, 149.

5. Morgan, W. T. J. and King, H. K., *Bioch. J.*, 1943, v37, 640.

6. Landy, M. and Batson, C., *J. Immunol.*, 1949, v62, 4.

† Granular mucin type 170W—Wilson Laboratories.

TABLE III. Resistance Lowering Properties of Hog and Human Mucin and Fractions Tested by Intraperitoneal Inoculation of Mice.

Inoculum Streptococcus group C, ml	Suspended in: Hog gastric mucin, crude	A_2 from hog mucin	Human mucin, crude	N_1 from human	N_2 from human	N_3 from human	Saline
10^{-6}	5/5*	5/5	5/5	0/5	—	5/5	0/5
10^{-7}	4/5	5/5	4/5	0/5	3/5	1/5	0/5
10^{-8}	0/5	2/5	1/5	0/5	3/5	0/5	0/5

* Fraction = No. dead at 4 days/No. inoculated. Human blood group A inhibiting titers A_2, <.001 γ; N_1, .02 γ; N_2, .09 γ; N_3, .37 γ. Mucin and fractions alone were not lethal.

TABLE IV. Resistance Lowering Properties of Human Respiratory Tract Mucin and Fractions Tested by Intraperitoneal Inoculation of Mice.

Inoculum Streptococcus group C, ml	Suspended in: Crude mucin	M_1	M_2	M_3	M_4	Saline
10^{-6}	5/5*	5/5	5/5	5/5	3/5	0/5
10^{-7}	4/5	5/5	4/5	3/5	1/5	0/5
10^{-8}	3/5	3/5	1/5	2/5	0/5	1/5

* Fraction = No. dead at 4 days/No. inoculated. Human blood group A inhibiting titers M_1, .0025 γ; M_2, .02 γ; M_3, .32 γ; M_4, 5.0 γ. Mucin and fractions alone were not lethal.

and dried from the frozen state. The supernate from this latter step is adjusted to a higher ethanol concentration and the resulting precipitate recovered and treated as above. The commercial hog gastric mucin was extracted after first homogenizing with the 90% phenol in a Waring blendor. The human mucin material, after sterilization with heat, as described above, was dried in vacuum and then suspended in 90% phenol as done with the hog gastric mucin. Each fraction obtained from both sources of material was, after washing with alcohol (95% ethanol), dialyzed against distilled water. The dialysis was carried out for 72 hours, water being changed every 24 hours. In the "N" series of fractions from human mucin, the amount of material obtained on adjustment of the supernatant to 10% ethanol was so small that in the subsequent series of fractions, series "M," the second fraction was obtained by adjustment of the supernatant to 33% ethanol. Two other fractions, besides that insoluble in 90% phenol and the "M"-2 fraction, were obtained by adjustment of the respective supernates to 55% and 75% ethanol respectively. These fractions were tested for resistance lowering effect by injecting 0.25 ml containing 10^{-6}, 10^{-7}, or 10^{-8} ml of the test culture intraperitoneally. The experiment included inoculations of organisms suspended in saline, 5% hog gastric mucin "A" fraction, 3% human mucin or fractions N_1, N_2, and N_3. Hog gastric mucin and human mucin alone were included as controls.

Table III records the results of these experiments. It will be noted that fraction N_3 of human mucin and A_2 of hog material were particularly effective in lowering resistance. The virulence enhancing factors in hog gastric mucin according to Morgan and King(5), Landy and Batson(6), are characterized by a strong blocking action of anti-human blood antigen A. Hemagglutination inhibition tests similar to the one described by Landy and Batson(6) were carried out on hog gastric mucin, human mucin, and their fractions. There was at least 300 times less blood group A substance in the N_3 fractions than in the A_2 fractions; yet both were active in lowering resistance. Also, the N_1 fraction with ten times more A substance than N_3 was devoid of resistance lowering properties, while N_3 material was active in this respect.

Table IV presents the results of tests on resistance lowering properties of the fractions. Fractions M_1, M_2, and M_3 were particularly active in lowering resistance. Again, there was no correlation between iso-hemagglutination inhibition and resistance lowering proper-

ties of the mucin fraction.

Summary. 1. Human respiratory tract mucin possesses a resistance lowering factor similar in activity to that of hog gastric mucin. This effect was shown by intrabronchial inoculation of rats with pneumococci suspended in saline or the two types of mucin. It was also shown by intraperitoneal inoculation of mice with streptococci suspended in saline, in two types of mucin and in fractions thereof. 2. Evidence against resistance lowering properties of mucin depending on the presence of blood group A antigen was obtained by fractionating human respiratory tract mucin and testing the fractions for blood group A antigen and the resistance lowering properties of the fractions.

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Tissue Distribution, Accumulation and Elimination of the Isomers of Benzene Hexachloride. (18631)

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One of the properties of benzene hexachloride which makes it an effective agricultural insecticide is the relatively persistent action of residues of this material remaining after spraying or dusting. However, this property may give rise to a consumer hazard through the ingestion of foodstuffs contaminated by these residues. In order to evaluate the potential consumer hazard acute(1) and chronic (2) toxicity experiments have been conducted. It was noted that in single oral doses the toxicity of the isomers decreased in the order of gamma, alpha, delta and beta. However, in the chronic experiments it was observed that the beta isomer was the most toxic followed by alpha, gamma and delta. An explanation of this phenomenon was sought by investigating the tissue distribution, the relative degree of accumulation after feeding diets containing the various isomers, and the rate of elimination of the isomers from the fat after removal of the benzene hexachloride from the diet. Early work from this laboratory on the distribution of the gamma isomer indicated that it might be stored preferentially in the fat(3).

Method. Rats used in this study were from our stock of Osborne-Mendel strain and they were fed *ad libitum* a diet consisting of ground commercial laboratory chow to which had been added the various isomers of benzene hexachloride dissolved in a small quantity of corn oil. The analytical method employed was that of Davidow and Woodard which depends upon the conversion of the benzene hexachloride to 1,2,4-trichlorobenzene and the determination of the latter by its characteristic ultraviolet spectrum(4). To determine the tissue distribution of benzene hexachloride, weanling rats were placed on diets containing 800 p.p.m. of the alpha, gamma and delta isomers, and 100 p.p.m. of the beta isomer. Feeding of these diets was continued for approximately 20 months after which time the rats were sacrificed and the tissues removed. Dogs weighing from 11 to 16 kg were given gelatin capsules of corn oil solutions containing 5% of the alpha isomer, 2% of the gamma isomer, or 10% of the delta isomer. Due to

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1. Woodward, G., and Hagan, E. C., *Fed. Proc.*, 1947, v6, 386.

2. Fitzhugh, O. G., Nelson, A. A., and Frawley, J. P., *J. Pharm. and Exp. Therap.*, 1950, v100, 59.

3. Laug, E. P., *J. Pharm. and Exp. Therap.*, 1948, v93, 277.

4. Davidow, B., and Woodard, G., *J. Assoc. Off. Agri. Chem.*, 1949, v32, 751.